

EXHIBIT A

Supporting Experimental Data

We determined whether the three candidate peptides, shown in Table 1, would attenuate A β 's SOD-like activity in a DCF assay and additionally determined the species specific nature of this modification.

Table 1 Identification of phage display peptides that bind human A β 1-42

Peptide sequence/length Name	Number of identical sequences after Round 4	Number of identical sequences after Round 5
TNPNNRRNTPQMLKR [#] 15-mer Peptide A (SEQ ID NO:3)	3/20	5/20
PLPQML [#] 6-mer Peptide B (SEQ ID NO:2)	7/20	8/20
MTMPTM 6-mer Peptide C (SEQ ID NO:1)	2/20	5/20

[#] Share sequence homology.

To achieve this, Peptide A (SEQ ID NO:3) (Figure 1a), Peptide B (SEQ ID NO:2) (Figure 1b) and Peptide C (SEQ ID NO:1) (Figure 1c) were added to 200 nM of each A β species (human A β 1-42, rat A β 1-42 and human reverse A β 42-1) in the presence of 400 nM Cu²⁺ at concentrations of 100, 200, 400, 1000 and 2000 nM.

The addition of Peptide A (Figure 1a) significantly and specifically reduced H₂O₂ production by human A β 1-42 (One way ANOVA; $F_{(5,84)} = 15.653$, $P < 0.001$), but had no effect on either human reverse A β 42-1 or rat A β 1-42. Additionally, it produced negligible native H₂O₂. Significant reductions in H₂O₂ levels were observed at 100, 200 and 400 nM ($P < 0.001$) and to a lesser extent at 1000 nM ($P < 0.05$). Similar results were found for Peptides B and C. The above findings, taken together, indicate that all three candidate peptides possess the ability to modify A β 1-42's SOD-like activity. However, of these, Peptide A is the most promising, based on its efficacy and specificity in reducing human A β 1-42's SOD-like activity.

We also determined whether the three candidate peptides protected against A β -induced neuronal toxicity as assessed by the LDH and MTT assays. The LDH assay measures the activity of LDH enzyme released from cells as a result of comprised plasma membrane integrity. The LDH release induced by A β 1-42 action on M17 cells was effectively attenuated in the presence of Peptide A at concentrations ranging from 5-100 μ M. (Figure 2a; One way ANOVA; $F_{(5,53)} = 30.847$, $P < 0.001$). Peptide A completely blocked A β 1-42 induced LDH release at a concentration of 50 μ M. Neither of the two 6-mer peptides significantly reduced

LDH levels as compared to A β only (Figure 2; One way ANOVA; MTM, $F_{(4,45)} = 1.126$, $P = 0.356$; PLP, $F_{(4,45)} = 3.710$, $P < 0.05$). Peptides B and C did not alter A β 1-42 induced LDH release at the concentrations tested (Fig 2b and 2c respectively).

We then further assessed the ability of the most promising peptide, Peptide A, to protect against A β -induced toxicity using the MTT assay. The MTT assay is commonly used to determine viability and/or metabolic activity of cells. Assessing the effect of the candidate peptides on A β toxicity in this assay is particularly relevant, as it has been shown that the addition of catalase (an enzyme that consumes H₂O₂) blocks A β -induced neurotoxicity, demonstrating that H₂O₂ is directly responsible for A β 's toxic effects in this assay.

M17 cells incubated for 6 h with 10 μ M human A β 1-42 in Locke's buffer resulted in a significant loss of viability/metabolic activity when compared to untreated controls. The addition of Peptide A at concentrations ranging from 1-100 μ M, to human A β 1-42 treated cells produced a dose-dependent increase in the percentage of metabolically active cells (Figure 3; results up to 5 μ M shown). These results indicate that this peptide can effectively block A β 1-42 induced neurotoxicity in the M17 cells. Similar experiments were carried out on Peptides B and C (data not shown). However, whilst there was some effect from these peptides, overall they were found to be much less effective.

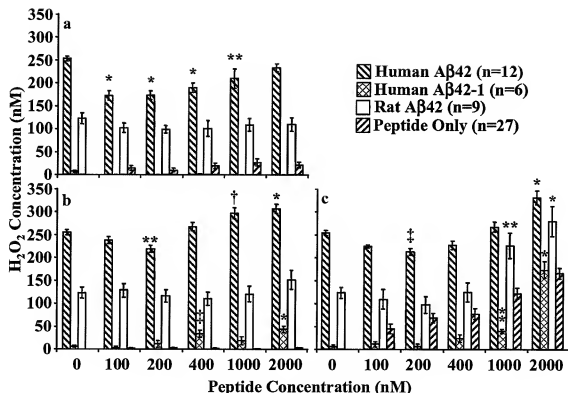


Figure 1. Dose-response effect of the three candidate peptides, Peptide A (a), Peptide B (b), and Peptide C (c), on H₂O₂ production by Aβ42 species. Data are presented as means ± SEM. For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. *p < 0.001, ** p < 0.05, † p < 0.005 and ‡ p < 0.01, significantly different compared to the respective Aβ species only (i.e. no peptide).

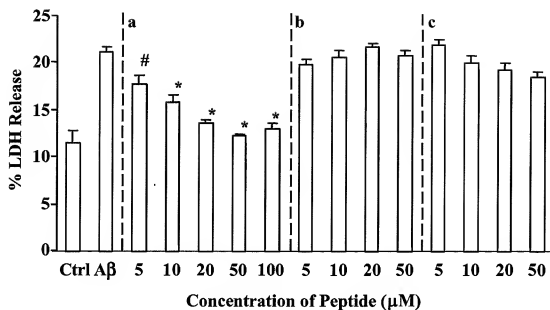


Figure 2. Percentage of lactate dehydrogenase (LDH) activity released by untreated and treated M17 cells. Cells were treated with 10 μ M human A β 1-42, for 120 hours, in the absence or presence of varying doses of the three candidate peptides, Peptide A (a), Peptide C (b), Peptide B (c). Data are presented mean \pm SEM (n = 9). For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. *p < 0.001 and #p < 0.01, significantly different compared to A β 1-42 only treated cells.

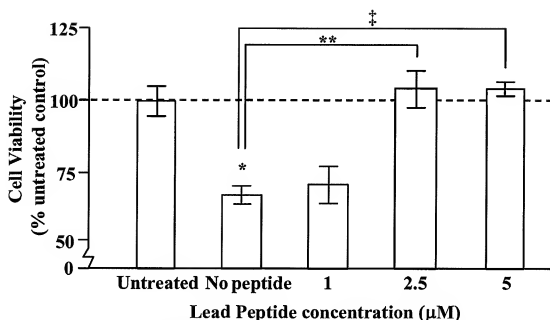


Figure 3 Cell viability of M17 cells treated with human A β 1-42 in the presence of the most promising peptide, Peptide A. M17 cells were treated for 6 hours at 37°C with soluble human A β 1-42 (10 μ M) in the absence or presence of Peptide A (1, 2.5 or 5 μ M). 10 μ M soluble human A β 1-42 in Lockes buffer results in a 35% reduction of cell viability. Data is expressed as a percentage of untreated controls and represents SEM of three experiment performed in sextuplicate (n=18). The addition of the Peptide A, significantly inhibited toxicity induced by soluble human A β 1-42 providing maximum protection at a concentration of 2.5 μ M. Data are presented as means \pm SEM. For statistical analyses, One-way ANOVA with post-hoc analysis (Tukey HSD), correcting for multiple comparisons, was performed. ** $P < 0.05$, † $P < 0.01$ versus No peptide, * $P < 0.001$ versus untreated.

Supplemental Data – Part 2

Acute A β Induced Neurodegeneration Rat Model

A reliable animal model of neurodegeneration may be induced by injections of A β 42 into the rat hippocampus. This A β model of Alzheimer's disease is an ideal tool for the *in vivo* investigation of compounds with neuroprotective potential.

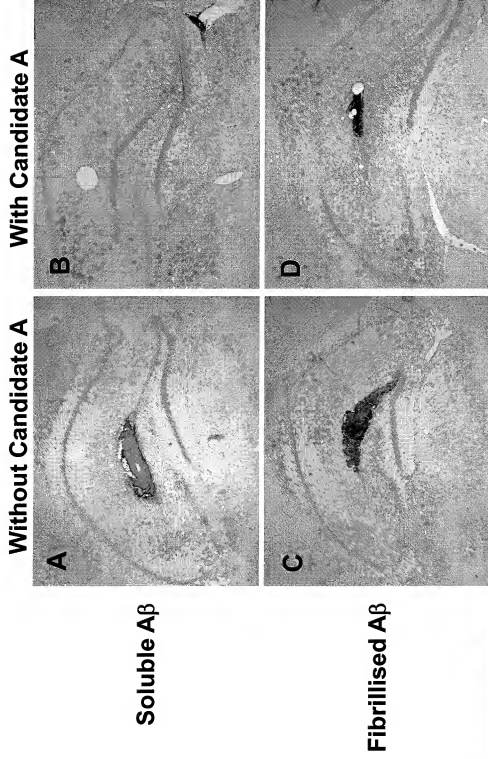
Injections of A β 42 induce degeneration of neurons of the hippocampal CA1 subfield, and produce neurochemical and immunohistochemical changes resembling those occurring in Alzheimer's disease [Kowall et al., PNAS USA. (1991); Miguel-Hidalgo et al., Eur Neuropsychopharmacol. (1998); Alvarez et al., J Neurosci. (1998)].

The present data results from an experiment to determine the effect of treatment of such a model with Candidate molecule A (15-mer: Thr-Asn-Pro-Asn-Arg-Arg-Asn-Arg-Thr-Pro-Gln-Met-Leu-Lys-Arg).

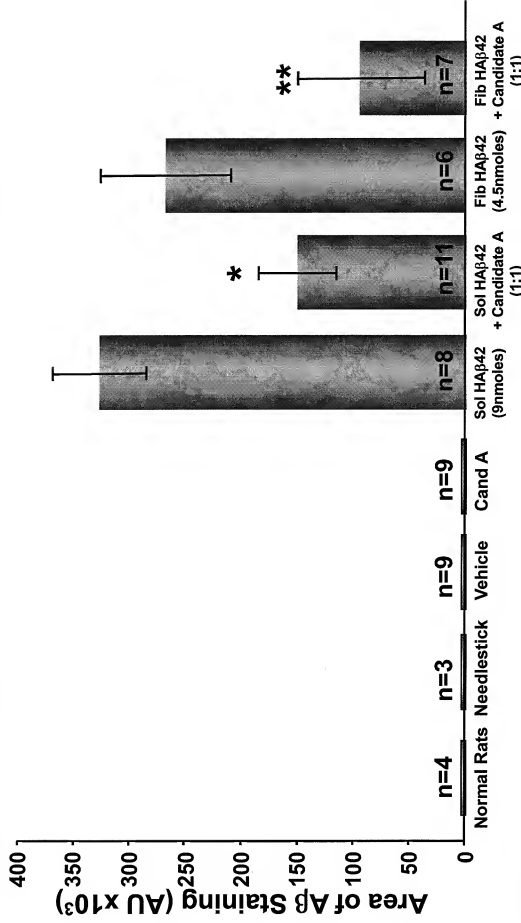
It was found that 15mer increases the clearance of A β from the brain, suggesting that the peptide has two modes of action:

1. neuroprotection by prevention of A β toxicity; and
2. facilitating A β clearance from the brain.

A β Staining of Rat Brain Injected With Human A β 42 \pm Candidate A Peptide



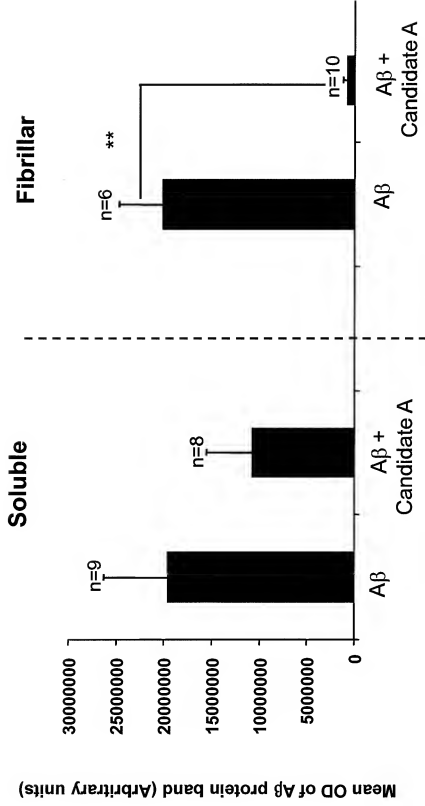
Levels of A β Staining in the Hippocampus of Rats



ANOVA: $F_{(3,28)}=5.271$, $P<0.005$; * $t=-2.957$, $p<0.05$ vs Soluble HAP42, ** $t=-2.427$, $p<0.05$ vs Fibrillar HAP42
[†]Bonferroni correction applied for multiple comparisons

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A β Levels in the Hippocampus of Injected Rats



*p<0.01 Significantly decreased from fibrillar A β by one way ANOVA with post-hoc (Tukey HSD)

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